REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

DNA ligases, as an essential component of DNA replication, recombination, and repair systems found from viruses to humans, catalyze the formation of a phosphodiester bond at single-stranded breaks on duplex DNA. DNA ligases can be classified into two families based on cofactor dependence. ATP-dependent ligases are found in bacteriophages, *Chlorella* virus PBCV-1, Vaccinia virus, Archea, yeasts, mammalian cells, and, more recently, eubacteria. NAD^+(i.e,. nicotinamide adenine dinucleotide)-dependent ligases, however, are found exclusively in eubacteria. While some higher eukaryotic organisms may use multiple ATP (i.e., adenosine triphosphate)-dependent ligases to fulfill diverse biological functions, some simple eubacteria genomes could host both an NAD^+-dependent ligase and an ATP-dependent ligase. The origin of the additional ATP-dependent ligases in these genomes remains to be determined.

Although the ATP-dependent ligases and NAD^+-dependent ligases share little sequence homology, all the ligases investigated so far use the same KXDG motif to form adenylated enzyme intermediate. Furthermore, they seem to be organized by similar domains and structural folds. The diversity of ligase sequences is not only reflected by their different optimal reaction conditions and kinetic rates, but, more importantly, by their different specificities toward match and mismatch substrates. Among the viral ATP-dependent ligases, the broad substrate tolerance is represented by the T4 enzyme which seals various mismatches on both the 3' and 5' side of the nick junction. Vaccinia ligase ligates various mismatches at both 3'-hydroxyl or 5'-phosphate sides with the exception of purine-purine mismatch pairs at the 3'-hydroxyl side. Mammalian ATP-dependent ligases show different substrate sensitivity, as ligase I is more sensitive to 3' mismatches than ligase III. Additionally, both ligase I and III tolerate a 3'C/T mismatch more than a 3'G/T mismatch. Little is known about archeal ATP-dependent ligases which may reveal the nature of the progenitor of ATP-dependent ligases. Studies on NAD^+-dependent DNA ligase from *E. coli*, along with T4 ligase, have contributed immensely to understanding of the basic biochemical pathway of the DNA ligation reaction. Studies on the NAD^+-dependent ligase from *Thermus thermophilus* HB8 have revealed the highly discriminative power this enzyme possesses.

Although mismatches at 5'-phosphate side are tolerated to some degree (5'A/C, 5'A/A, 5'C/A, 5'C/T, 5'G/T, 5'G/A, 5'T/T, 5'T/G), mismatches at the 3'-hydroxyl side essentially abolish nick-closure activity except 3'G/T or 3'T/G mismatch. Apparently, sequence divergence and subsequent subtle structural variation among DNA ligases underlie an enzyme's recognition preferences toward different mismatched base-pairs.

The study of ligase biochemistry is not only important for understanding its biological functions, but also for developing new technologies. The single nucleotide discrimination observed on DNA ligases has led to the development of ligase-mediated detection techniques. Ligase-based linear signal amplification known as LDR (i.e., ligase detection reaction), combined with PCR (i.e. polymerase chain reaction)-based gene specific target amplification, has been proven to be a powerful tool in cancer and disease gene mutation detection. PCR/LDR technique relies on two properties of a DNA ligase: (i) specificity and (ii) thermostability. *Tth* (i.e., *Thermus thermophilus* HB8) DNA ligase has been successfully used in LDR and LCR (i.e. ligase chain reaction) due to its highly discriminative nick closure activity toward a perfect match substrate and its thermostability which makes thermocycling possible. To date, one more ligase was cloned and sequenced from *T. scot.* (i.e., *Thermus scotoductus*), but the substrate specificity of this ligase was not determined.

Despite the existence of a number of ligases from different host sources, the need remains to identify additional ligases with greater fidelity. The present invention is directed to achieving this objective as a result of the cloning and expression of a ligase from *T. sp.* (i.e., *Thermus* species) AK16D and the biochemical characterization of this high fidelity enzyme.

The above amendments to the specification reflect the addition of sequence identifiers as required for compliance with 37 C.F.R. §§ 1.821-825, and the correction of minor typographical errors. In addition, the protein sequences corresponding to SEQ. ID. NOs. 25-31 have been added to the sequence listing. Because SEQ. ID. NOs. 25-31 were disclosed in the specification as GenBank Accession Nos. AF092863 (copy attached hereto as Exhibit 1), AF092864 (copy attached hereto as Exhibit 2), AF092865 (copy attached hereto as Exhibit 3), AF092866 (copy attached hereto as Exhibit 4), AF092867 (copy attached hereto as Exhibit 5), AF092868(copy attached hereto as Exhibit 6), and 1085749 (copy attached hereto as Exhibit 7), respectively, applicants submit that no new matter has been added.

The rejection of claims 1-15 under 35 U.S.C. § 101 for failure to claim statutory subject matter is respectfully traversed in view of the above amendments.

The rejection of claims 1-8 and 10-15 under 35 U.S.C. § 112 (1st para.) for failure to meet the written description requirement is respectfully traversed in view of the above amendments.

The rejection of claims 1-8 and 10-15 under 35 U.S.C. § 112 (2nd para.) for lack of enablement is respectfully traversed in view of the above amendments.

The rejection of claims 1-3 and 8 under 35 U.S.C. § 102(b) as anticipated by Luo et al., "Improving the Fidelity of *Thermus thermophilus* DNA Ligase," *Nucleic Acids Research* 24(14):3071-3078 (1996) ("Luo") is respectfully traversed in view of the above amendments.

Luo teaches two mutant ligases of *Thermus thermophilus* ("*Tth*"), K294R and K294P, having increased ligation fidelity over wild type *Tth* ligase. As amended, claim 9 of the present invention is drawn to "[a]n isolated thermostable ligase, wherein the thermostable ligase has an amino acid sequence of SEQ. ID. NO: 1." Luo does not teach or suggest a thermostable ligase having SEQ. ID. NO: 1 and, therefore, cannot anticipate claim 9.

In addition, claim 48 is drawn to "[a]n isolated thermostable ligase, wherein the ligase is a mutant of a wild-type thermostable ligase having a histidine adjacent a KXDG motif, wherein the mutant thermostable ligase has a mutation in its amino sequence where the histidine adjacent the KXDG motif in the wild-type thermostable ligase is replaced with an arginine, and wherein X is any amino acid." Luo does not teach or suggest an isolated ligase having a histidine adjacent a KXDG motif. Therefore, Luo does not anticipate claim 48.

New claim 50 is drawn to "[a]n isolated mutant thermostable ligase, wherein the thermostable ligase is encoded by a DNA molecule having a nucleotide sequence of SEQ. ID. NO: 2." Since Luo does not teach or suggest a mutant thermostable ligase having a nucleotide sequence of SEQ. ID. NO: 2, it cannot anticipate claim 50.

For all these reasons, the rejection, under 35 U.S.C. § 102(b), based on Luo alone should be withdrawn.

The rejection of claims 5, 10-11, and 15 under 35 U.S.C. § 102(b) as anticipated by Luo as evidenced by Tong et al., "Biochemical Properties of a High Fidelity DNA Ligase from *Thermus* species AK16D," *Nucleic Acids Research* 27:788-794 (1999) ("Tong"), is respectfully traversed in view of the above amendments.

Tong discloses the cloning and expression of the *Thermus* species AK16D ligase which has a 6-fold higher ligation fidelity than wild-type *Tth* ligase using Mg^{2+} as the divalent ion, but reduced ligation fidelity when Mn^{2+} is substituted for Mg^{2+}. Tong is cited by the U.S. Patent and Trademark Office as evidentiary support for the inherent property of improved fidelity of the mutant DNA ligase taught by Luo. However, claims 5, 10-11, and 15 have been canceled by the above amendments and the pending claims are not directed to improved fidelity limitations.

Therefore, the rejection of claims 5, 10-11, and 15 under 35 U.S.C. § 102(b) as anticipated by Luo as evidenced by Tong should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450, on the date below.

May 4, 2004 — Jo Ann Whalen
Date — Jo Ann Whalen